

## BIOGRAPHICAL SKETCH

NAME <b>Mark S. Shapiro</b>		POSITION TITLE Professor of Physiology	
eRA COMMONS USER NAME shapiro			
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
University of Chicago, Chicago, IL	B. A.	1980-1984	Physics
Rush University, Chicago, IL	Ph.D.	1986-1991	Physiology
University of Washington, Seattle, WA	Postdoctoral	1991-2000	Physiology/Biophysics

### A. PERSONAL STATEMENT

My research program has spanned the physiology and modulation of voltage-gated  $K^+$  and  $Ca^{2+}$  channels in neurons, cardiomyocytes and non-excitable cells. We have focused on voltage-gated “M-type” (KCNQ)  $K^+$  and  $Ca^{2+}$  channels, and signaling pathways of  $G_{q/11}$ -coupled receptors, using patch-clamp electrophysiology of native neurons and heterologous systems, biochemistry, confocal and TIRF microscopy, molecular biology and live single-cell and whole-animal imaging. Our major publications have shown the  $PIP_2$  sensitivity of both types of channels, the mechanisms and structural determinants of receptor-mediated suppression of  $I_M$  and  $I_{Ca}$ , the modulation of KCNQ channels by calmodulin, A-kinase anchoring proteins (AKAPs) and Src kinase, the roles of M channels in airway smooth muscle, and in sensory neurons. We also seek to systematically explore the role of AKAP79/150 in orchestrating transcriptional and regulatory control of M/KCNQ channels in sympathetic and nodose sensory neurons. We also use STORM super-resolution nanoscopy to probe the multi-protein complexes underlying modulation of ion channels. We are also focusing on novel strategies to prevent acquired epilepsies, and the role of M channel regulation in epileptogenesis. Additionally we also are exploring novel and provocative roles of M/KCNQ channels as a neuroprotective mechanism during cerebrovascular ischemic stroke and Traumatic Brain Injury, together with other researchers here in San Antonio, to prevent the development of epilepsy and pathological co-morbidities. As former Chairman of the Physiology governing committee, and discipline member of the Physiology/Pharmacology, Biochemistry and Biophysics and Neuroscience tracks in our Ph.D. program, I am committed to graduate education and in mentoring our pre- and post-doctoral trainees.

### B. POSITIONS AND HONORS:

1986-91	Predoctoral Research Assoc., Department of Physiology, Rush University, Chicago, IL; Advisor: Thomas E. DeCoursey
1991-95	Senior Fellow, Department of Physiology & Biophysics, Laboratory of Bertil Hille, University of Washington, Seattle, WA
1995-97	Senior Fellow, Department of Physiology & Biophysics, Laboratory of William Zagotta, University of Washington, Seattle, WA
1997-98	Senior Fellow, Department of Anesthesiology, Laboratory of Kenneth Mackie, University of Washington, Seattle, WA
1998-00	Senior Fellow, Department of Anesthesiology, Laboratory of Kenneth Mackie, and Department of Physiology & Biophysics, Laboratory of Bertil Hille, University of Washington, Seattle, WA
2000-05	Assistant Professor, Department of Physiology, UT Health Science Center, San Antonio, TX
2005-10	Associate Professor, Department of Physiology, UT Health Science Center, San Antonio, TX
2010-	Professor, Department of Physiology, UT Health Science Center, San Antonio, TX
2014-	Professor, cross-appointment in the Department of Neurosurgery. UTHSCSA

### HONORS AND OTHER PROFESSIONAL ACTIVITIES:

2004-	Medical Research Council (equivalent of NIH), United Kingdom, Grant Reviewer, ad hoc United States/Israel Bi-national Science Foundation, Grant Reviewer, <i>ad hoc</i>
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2005- Israeli National Science Foundation, Grant Reviewer, *ad hoc*  
 2006-10 Study Section Member, National Institutes of Health, BPNS IRG  
 2008 National Institutes of Health Site Visit, Committee Member  
 2009 Reviewer, Wittgenstein Award, Austrian Science Fund  
 2013-16 President's Council Faculty Scholar, UTHSCSA  
 2015-17 President, Society of General Physiologists.

**Editorial Boards:** J. Neuroscience, Associate Editor (2012-), Pflügers Archiv (2008-11), Journal of Biological Chemistry (2009-14), PLoS One (2012-), Channels, 2011-

## C. CONTRIBUTIONS TO SCIENCE:

**1. Shapiro, M.S., J.P. Roche, E.J. Kaftan, H. Cruzblanca, K. Mackie and B. Hille.** (2000). Reconstitution of muscarinic modulation of the KCNQ2/KCNQ3 K<sup>+</sup> channels that underlie the neuronal M current. *Journal of Neuroscience* 20:1710-1721.

This paper as a post-doctoral fellow in the Hille lab represented the first reconstitution of the classical muscarinic suppression of the M current, using the cloned KCNQ2 and KCNQ3 channel, and muscarinic M<sub>1</sub> receptor gene products that had just been identified as underlying the neuronal M current. It launched my career as an independent PI.

**2. Gamper, N. and M.S. Shapiro.** (2003) Calmodulin mediates Ca<sup>2+</sup>-dependent modulation of M-type K<sup>+</sup> channels. *Journal of General Physiology* 122:17-31.

This paper was the first to show that calmodulin (CaM) is the Ca<sup>2+</sup> sensor modulating M-type channels in neurons via IP<sub>3</sub>-mediated Ca<sup>2+</sup> signals. We went on to show the mechanism of receptor specificity in such Ca<sup>2+</sup>/CaM-dependent M-current modulation (**Zaika et al., 2007**), and the interaction between apoCaM and Ca<sup>2+</sup> CaM with KCNQ2-5 channels using Förster resonance energy transfer (FRET) under total internal reflection fluorescence (TIRF) illumination (**Bal et al., 2008**). We are now investigating the structural rearrangements of the channel/CaM complex underlying this action using cutting-edge biochemical techniques.

**3. Hernandez, C.C., Zaika, O. and M.S. Shapiro** (2008). A carboxy-terminal inter-helix linker as the site of phosphatidylinositol 4,5-bisphosphate action on Kv7 (M-type) K<sup>+</sup> channels. *Journal of General Physiology*. 132: 361-381.

This paper was the first to show the site of KCNQ2 and KCNQ3 M-type channels by phosphoinositides (PIP<sub>2</sub>) using single-channel recordings from inside-out patches and molecular modeling. We identified a “cationic cluster” of basic residues between the A&B helices in the carboxy terminus as a major site of PIP<sub>2</sub> interactions. This work was made possible by a previous paper (**Li et al., 2004**), which was the first to show the direct apparent affinity of PIP<sub>2</sub> for KCNQ2-4 channels using single-channel inside-out patches. Using other cutting-edge approaches, we are now investigating the other putative sites suggested to be domain interacting with PIP<sub>2</sub> by other groups (Choveau et al., 2016, *Journal of General Physiology*, under revision).

**4. Zhang, J., Bal, M, Zaika, O., and M. S. Shapiro** (2011). AKAP79/150 signal complexes in G-protein modulation of neuronal ion channels. *Journal of Neuroscience* 31:7199-7211.

This paper established the receptor-specific clustering of M channels, the scaffold protein, AKAP79/150, and certain G<sub>q/11</sub>-coupled receptors, in neurons. This work was preceded by our demonstration of the disruption of AKAP79/150-KCNQ channels complexes by Ca<sup>2+</sup>/CaM, but not apoCaM (**Bal et al., 2010**). We are now investigating such complexes at the single-complex level in a variety of neuronal types using STORM super-resolution microscopy (Zhang et al., 2016, under revision for Science).

**5. Zhang, J. and M. S. Shapiro** (2012). Activity-dependent Transcriptional Regulation of M-type K<sup>+</sup> Channels by AKAP79/150-mediated NFAT Actions. *Neuron* 76:1133-46.

This landmark paper reported the discovery of a novel signaling pathway involving AKAP79/150, calcineurin (CaN) and L-type (Ca<sub>v</sub>1) voltage-gated Ca<sup>2+</sup> channels in upregulation of KCNQ2 and KCNQ3 transcription resulting from neuronal activity in peripheral ganglia. We also showed these M-channel genes to be profoundly transcriptionally up-regulated in the hippocampus following a single chemoconvulsant seizure, leading to the hypothesis of this cytoplasmic/nuclear signaling pathway as representing a homeostatic negative feedback mechanism limited seizure-induced epileptogenesis in the brain. We are currently investigating such a hypothesis using brain-slice electrophysiology, transgenic mice and transcriptional analyses.

**6. Zhang, J., Carver, C., Bierbower, S.B., Lechleiter, J.D. and M. S. Shapiro.** (2016). Clustering and functional coupling of diverse ion channels and signaling proteins revealed by super-resolution STORM microscopy in neurons. *Neuron* (*in press*).

Using cutting-edge STORM super-resolution nanoscopy, which is revolutionizing the field of visible-light imaging, this work investigates how scaffold proteins, such as A-kinase Anchoring Protein (AKAP)79/150,

which are key to spatiotemporal focusing of directed signals in all parts of the nervous system (and beyond), organizes diverse and distinct ion channels and receptors in neurons clustered together in nanodomains, including strong evidence of their functional interaction in primary neurons. This work unambiguously demonstrates the intimate physical association of AKAP79/150 with “M-type” (KCNQ, Kv7) K<sup>+</sup> channels, TRPV1 “pain-sensing” cation channels and Cav1.2 (L-type) voltage-gated Ca<sup>2+</sup> channels, which are essential to control of neuronal excitability, nociception and the “excitation/transcription” coupling that is fundamental to neuronal plasticity

#### D. CURRENT RESEARCH SUPPORT:

Department of Defense, CDMRP, (PI: M. S. Shapiro) 10/1/15-9/30/18. *Novel Strategies Targeting Signaling Molecules of Neurons and Astrocytes to Prevent Acquired Epilepsies.*

Direct/Indirect/Total costs : \$780,003/\$375,705/\$1,155,708

Grant-in-Aid, American Heart Association (PI: M.S. Shapiro) 7/1/15-6/30/17. *Dual novel approaches targeting neurons and astrocytes for protection from brain injury after cerebrovascular stroke.*

Total Direct/Indirect/Total costs: \$63,000/\$7,000/\$70,000/year

2 R01 NS043394, National Institutes of Health (PI: M. S. Shapiro) 6/01/02 - 5/31/15, NCE until 5/31/16  
*Mechanism and functional role of lipid modulation of neuronal ion channels.*

Direct costs/Indirect costs/Total in current funding period: \$283,668/\$125,775/\$1,631,970

R01NS094461, National Institutes of Health (PI: M. S. Shapiro) 09/30/15 - 07/31/20  
*Clustering of individual and diverse ion channels together into complexes, and their functional coupling, mediated by A-kinase anchoring protein 79/150 in neurons*

Direct costs/Indirect costs/Total in current funding period: \$218,750/ \$111,628/ \$330,378

#### SEMINARS AND INVITED SPEAKER PRESENTATIONS (recent):

- 2011 Invited speaker, *Trends and challenges in ion channel research*, Tenerife, Canary Islands, Spain.  
Invited symposium speaker, Experimental Biology Meeting, Washington, DC  
Invited speaker, 3<sup>rd</sup> Ion Channel Conference: “Ion Channels: Structure, Function & Therapeutics”, Shanghai, China.
- 2012 Department of Pharmacology, University of Iowa Carver School of Medicine, Iowa City, IA.  
University of Texas at Austin, Section of Neurobiology, Austin, TX.  
Invited speaker and session chair, Cold Spring Harbour Laboratory meeting, “Ion Channels: Biophysics, Diseases and Therapeutics,” Suzhou, China.  
Department of Pharmacology, Peking University, Beijing, China.  
Department of Pharmacology, Hebei Medical University, Shijiazhuang, China.
- 2013 Department of Pharmacology, University of California at Irvine, Irvine, CA.  
Department of Biochemistry, State University of New York at Buffalo, Buffalo, NY.
- 2014 Department of Neuroscience, Thomas Jefferson University, Pennsylvania, PA.  
Invited Speaker, Ukrainian Neuroscience Society Meeting, Kiev, Ukraine  
Invited Speaker, Physiological Society, London, UK  
Invited Speaker, University of Florida, Department of Neuroscience
- 2015 Invited Speaker, University of Illinois at Urbana/Champaign, Dept. of Mol. and Integrative Physiol.
- 2016 Invited Speaker, Ion channels Gordon Conference, Mt Holyoke, Massachusetts.  
Invited Speaker, University of California at Davis, Center for Neuroscience  
Invited Speaker, Medical College of Georgia, Augusta, GA  
Invited Symposium Organizer and speaker, Federation of Latin American Neuroscientists, Buenos Aires, Argentina.

#### BOOKS AND/OR BOOK CHAPTERS

- Shapiro, M.S.** and T.E. DeCoursey (1991). Chloride currents in bovine pulmonary artery endothelial cells. *In* Electrophysiology and Ion Channels of Vascular Smooth Muscle Cells and Endothelial Cells, edited by Nicholas Sperelakis, pp. 327-336.
- Gamper, N. and **M.S. Shapiro** (2006). Exogenous expression of proteins in neurons using the biolistic particle delivery system. *In* Methods in Molecular Biology: Ion Channels. (Humana Press, USA).

- Shapiro, M.S.** and J.D. Stockand, editors. Methods in Molecular Biology: Ion Channels. (Humana Press, USA).
- Gamper, N. and **M.S. Shapiro** (2009). Regulation of neuronal ion channels of sympathetic neurons. *In* Structure, Function and Pharmacology of Neuronal Voltage-Gated Ion Channels, Len Kaczmarek and Valentin Gribkoff, Eds.
- Shapiro, M.S.** (2011). Ion channel regulation by G-protein coupled receptors - recent advances with optical biosensors. *In* Encyclopedia of Biophysics, Gordon Roberts, Ed. (*in press*).
- Bierbower, S.M. and **M. S. Shapiro** (2013). Förster resonance energy transfer-based imaging at the cell surface of live cells. *In* Ion Channels. Methods and Protocols, Nikita Gamper, Ed. (Springer: New York).
- Gamper, N. and **M.S. Shapiro** (2015). KCNQ Channels. *In*: Handbook of Ion Channels, Jie Zheng and Matt Trudeau, eds. (CRC Press, Boca Rotan, FL).
- JOURNAL ARTICLES** (Selected from >70 peer-reviewed publications)
- Gamper, N., J.D. Stockand, and **M.S. Shapiro**. (2003). Subunit-specific modulation of KCNQ potassium channels by Src tyrosine kinase. Journal of Neuroscience 23:84-95.
- Gamper, N. and **M.S. Shapiro**. (2003) Calmodulin mediates  $\text{Ca}^{2+}$ -dependent modulation of M-type  $\text{K}^+$  channels. Journal of General Physiology 122:17-31.
- Li, Y., N. Gamper and **M.S. Shapiro**. (2004). Single-channel analysis of KCNQ  $\text{K}^+$  channels reveals the mechanism of augmentation by a cysteine-modifying reagent. Journal of Neuroscience 24:5079-5090.
- Shapiro, M.S.** Why biophysicists make models: Quantifying modulation of the M current. (2004). J. Gen. Physiol. 123:657-662.
- Li, Y., P. Langlais, N. Gamper, F. Liu and **M.S. Shapiro**. (2004). Dual phosphorylations underlie modulation of unitary KCNQ  $\text{K}^+$  channels by Src tyrosine kinase. Journal of Biological Chemistry 279: 45399-45407.
- Gamper, N., V. Reznikov, Y. Yamada, J. Yang and **M.S. Shapiro**. (2004).  $\text{PIP}_2$  signals underlie receptor-specific  $\text{G}_{q/11}$ -mediated modulation of N-type  $\text{Ca}^{2+}$  channels. Journal of Neuroscience 24(48):10980-10992.
- Li, Y., Gamper, N., Hilgemann, D.W. and **M. S. Shapiro**. (2005). Regulation of Kv7 (KCNQ)  $\text{K}^+$  channel open probability by phosphatidylinositol (4,5)-bisphosphate. Journal of Neuroscience 25(43):9825-35.
- Gamper, N., Y. Li and **M.S. Shapiro** (2005). Structural requirements for differential sensitivity of KCNQ  $\text{K}^+$  channels to modulation by  $\text{Ca}^{2+}$ /calmodulin. Mol Bio Cell 16:3538-3551.
- Delmas, P, Coste, B., Gamper, N. and **M.S. Shapiro**. (2005). Phosphoinositide lipid second messengers: New paradigms for calcium channel modulation. Neuron. 47:179-182.
- Zaika, O., Lara, L., Gamper, N., Hilgemann, D.W., and **M.S. Shapiro**. (2006). Angiotensin II regulates neuronal excitability via  $\text{PIP}_2$ -dependent modulation of Kv7 (M-type)  $\text{K}^+$  channels. J. Physiol. 575:49-67.
- Gamper, N., Zaika, O., Li, Y., Martin, P., Hernandez, C.C., Perez, M.R., Wang, A.Y.C., Jaffe, D.B. and **M.S. Shapiro**. (2006). Oxidative modification of M-type potassium channels as a mechanism of cytoprotective neuronal silencing. EMBO J. 25:4996-5004.
- Gamper, N. & **M.S. Shapiro**. (2007). Target-specific  $\text{PIP}_2$  signaling: how m it work? J. Physiol. 582: 967-75.
- Zaika, O.; Tolstykh, G.P.; Jaffe, D.B. and **M.S. Shapiro**. (2007).  $\text{IP}_3$ -mediated  $\text{Ca}^{2+}$  signals direct purinergic  $\text{P2Y}$ -receptor regulation of neuronal ion channels. J. Neurosci. 27:8914-8926.
- Gamper, N. and **M.S. Shapiro**. (2007). Regulation of ion transport proteins by membrane phospholipids. Nature Neuroscience Reviews. 8:921-34.
- Hernandez, C.C., Zaika, O, Tolstykh, G.P. and **M.S. Shapiro** (2008). Regulation of neural KCNQ channels: signaling pathways, structural motifs and functional implications. J Physiol. 586(7):1811-21.
- Bal, M., Zaika, O, Martin P. and **M. S. Shapiro** (2008). Calmodulin binding to M-type  $\text{K}^+$  channels assayed by TIRF/FRET in living cells. J. Physiol. 586(9):2307-20.
- Hernandez, C.C., Zaika, O. and **M.S. Shapiro** (2008). A carboxy-terminal inter-helix linker as the site of phosphatidylinositol 4,5-bisphosphate action on Kv7 (M-type)  $\text{K}^+$  channels. Journal of General Physiology. 132: 361-381.
- Bal, M., Zhang, J., Zaika, O, Hernandez, C.C. and **M.S. Shapiro** (2008). Homomeric and heteromeric assembly of KCNQ (Kv7)  $\text{K}^+$  channels assayed by TIRF/FRET and patch-clamp analysis. Journal of Biological Chemistry 283:30668-76.
- Zaika, O., Hernandez, C.C., Bal, M., Tolstykh, G.P. and **M.S. Shapiro**. (2008). Determinants within the turret and pore-loop domains of KCNQ3  $\text{K}^+$  channels governing functional activity. Biophysical Journal 95:1-17.
- Jeske, N.A., Patwardhan, A.M., Ruparel, N.B., Akopian, A.N., **Shapiro, M.S.** and M.A. Henry (2009). A-Kinase Anchoring Protein 150 Controls Protein Kinase C-mediated Phosphorylation and Sensitization of

- TRPV1. *Pain* **146**:301-7.
- Shapiro, M.S.** (2009). An ion channel hypothesis to explain divergent cardiovascular safety of COX-2 inhibitors: the answer to a hotly-debated puzzle? *Mol. Pharm.* 76:1-4.
- Hernandez, C.C., Falkenburger, B. and **M.S. Shapiro** (2009). Affinity for phosphatidylinositol 4,5-bisphosphate determines muscarinic agonist sensitivity of Kv7 K<sup>+</sup> channels. *Journal of General Physiology* 134:437-48.
- Bal, M., Hernandez, C.C., Zhang, J., Zaika O. and **M.S. Shapiro**. (2010). Ca<sup>2+</sup>/calmodulin disrupts AKAP79/150 interactions with KCNQ (M-type) K<sup>+</sup> channels. *Journal of Neuroscience* 30:2311-23.
- Zaika, O., Zhang J. and **M. S. Shapiro** (2011). Combined phosphoinositide and calcium signals mediating receptor specificity toward neuronal calcium channels. *Journal of Biological Chemistry* 286:830-41.
- Zhang, J., Bal, M, Zaika, O., and **M. S. Shapiro** (2011). AKAP79/150 signal complexes in G-protein modulation of neuronal ion channels. *Journal of Neuroscience* 31:7199-7211.
- Zaika, O., Zhang J. and **M. S. Shapiro** (2011). Functional role of M-type (KCNQ) K<sup>+</sup> channels in adrenergic control of cardiomyocyte contraction by sympathetic neurons. *J. Physiol.* 589 (10) 2559-2568.
- Chaudhury, S., Manjot Bal, M., **Shapiro, M. S.** and N. A. Jeske (2011). AKAP150-mediated TRPV1 sensitization is disrupted by Ca<sup>2+</sup>/calmodulin. *Molecular Pain.* 7:34.
- Klinger, F, Gould, G., Boehm, S and **M. S. Shapiro** (2011). Distribution of M-channel subunits KCNQ2 and KCNQ3 in rat hippocampus. *Neuroimage.* 58:761-769.
- Choveau, F.S., Hernandez, C.C., Bierbower, S.M. and **M. S. Shapiro** (2012). Pore determinants of KCNQ3 K<sup>+</sup> current expression. *Biophysical Journal* 102:2489-98.
- Choveau, F.S., Bierbower, S.M. and **M. S. Shapiro** (2012). Pore helix-S6 interactions are critical in governing current amplitudes of KCNQ3 K<sup>+</sup> Channels. *Biophysical Journal* 102:2499-2509.
- Choveau, F.S. and **M. S. Shapiro** (2012). Regions of KCNQ K<sup>+</sup> channels controlling functional expression. *Frontiers in Membrane Physiology and Biophysics* 3:1-6.
- Zhang, J. and **M. S. Shapiro** (2012). Activity-dependent Transcriptional Regulation of M-type K<sup>+</sup> Channels by AKAP79/150-mediated NFAT Actions. *Neuron* 76:1133-46.
- Evseev, A., Semenov, I., Medina, J., Dube, P., **Shapiro, M. S.** and R. Brenner (2013). Functional effects of KCNQ K<sup>+</sup> channel modifiers in airway smooth muscle. *Frontiers in Physiology.* 4:1-11.
- Bierbower, S.M. and **M.S. Shapiro** (2013). Förster resonance energy transfer-based imaging at the cell surface of live cells. *Methods Mol. Biol.* 998:209-16.
- Bierbower, S.M., Choveau, F., Lechleiter, J.D. and **M.S. Shapiro** (2015). Augmentation of M-type (KCNQ) potassium channels reduces stroke-induced brain injury. *J. Neuroscience.* **35**:2101-2111.
- Zhang, J. and **M.S. Shapiro**. (2015). Novel roles of AKAP79/150 in orchestrating multi-protein signaling complexes in brain and peripheral nerve. *J. Physiology* (epub Feb 4<sup>th</sup>, 201 Final pub: 2016;594(1):31-37.5).
- Choveau, F., and **M. S. Shapiro**. (2015). The helix C-D linker determines KCNQ3 current amplitudes by controlling channel trafficking. *PlosOne* 10(12):Dec. 21, e0145367.
- Choveau, F., Hernandez, C., C. and **M. S. Shapiro** (2016). Phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) regulates KCNQ3 K<sup>+</sup> channels through distinct sites of action (under revision to *Journal of General Physiology*).
- Zhang, J., Carver, C., Bierbower, S.B., Lechleiter, J.D. and **M. S. Shapiro**. (2016). Clustering and functional coupling of diverse ion channels and signaling proteins revealed by super-resolution STORM microscopy in neurons. *Neuron* Oct 19;92(2):461-478.

The Shapiro lab focuses on the physiology, regulation and functional role of ion channels of excitable cells, particularly neurons, in health and disease. Our lab features a “molecule to therapeutic” approach to science, in which the lab engages techniques running the gamut from biochemistry and structural biology (e.g., X-ray crystallography, NMR spectroscopy), immunochemistry, cellular and brain-slice patch-clamp electrophysiology from tissue-culture cells expressing cloned ion channels, receptors and signaling molecules, preparations of native neurons from peripheral ganglia and brain, a variety of cutting-edge imaging modalities (e.g.,  $\text{Ca}^{2+}$  and fluorescent protein reporter imaging, confocal and Total Internal Reflection Fluorescence (TIRF) microscopy of neurons and brain slices, super-resolution STORM nanoscopy), and behavioral assays using mouse models. This variety of approaches allows our lab to use molecular biology to alter, at will, cDNA plasmids, to assay transcriptional regulation, and to test our hypotheses by using molecular biology to mutate or tag cDNA constructs for the signaling molecules of interest, or by using transgenic or genetically-altered rodents.

The projects in the Shapiro lab are to investigate the signaling mechanisms acting on ion channels in neurons that regulate nervous function, including how nociceptor sensory neurons sense painful stimuli, the etiology of seizures and epilepsy (epileptogenesis), the regulation of activity in the hippocampus where new memories are thought to develop, the molecular architecture of cells and circuits in the brain and peripheral ganglia, and to develop novel therapeutic approaches to prevent brain damage after traumatic brain injury and stroke.

Specifically, the project focusing on sensory neurons seeks to identify and understand the function of multi-protein complexes in the plasma membrane of nociceptors that are mediated by scaffold protein called A-kinase Anchoring Protein 79/150 (AKAP79/150), and using super-resolution nanoscopy to image them at the single-complex level using visible light. The inventors of this “super-resolution” technique won the Nobel Prize for this in 2014. The signaling proteins that we examine are G-protein coupled receptors, ion channels, and kinases/phosphatases. This project has enormous implications for novel therapies for pain, which could stop pain at the sensory neurons, rather than dull those inputs into the brain using opioids, which have produced opiate addiction, overdoses and deaths around the world.

The two most translational projects are to test whether increasing the activity of a certain  $\text{K}^+$  ion channel found in almost all neurons, using novel pharmacological compounds, prevents excessive brain damage after either a stroke or a traumatic brain injury (TBI), as well as whether this novel therapy could prevent the subsequent cognitive, motor, and behavioral assays in living mice. We have a high-technology mouse stroke model in which we can induce a modest stroke using laser light in living mice. For the TBI project, the goals are to see if these  $\text{K}^+$  ion channel “openers” administered to the mice after a TBI can prevent the seizures and epilepsy that are commonly seen after TBI in people, as well as the similar brain damage described above, including behavioral assays for depression and social isolation. In this project, we employ two TBI mouse models, one that simulates the type of TBI often experienced by people in falls or car accidents, and a “blast model” at San Antonio Military Medical Center that well simulates the types of TBI experienced by men and women in the armed services in combat.

Another project uses techniques of biochemistry and structural biology, such as X-ray crystallography and Nuclear Magnetic Resonance Spectroscopy, to obtain the atomic structure of signaling proteins bound together at their sites of interactions. This involves protein purification from bacterial expression systems, biochemical analyses of the purified proteins, and a spectrum of such biochemical and structural experiments performed. Finally, we are probing how epileptogenesis occurs, in general, and how we could prevent it, using a variety of transgenic mice, brain-slice electrophysiology, and advanced optical techniques.

The Xiangya medical students in our lab would be free to engage any of these projects in consultation with Dr. Shapiro as PI. For all of these projects, there would be post-doctoral fellows and Ph.D. students to work with on a day-to-day basis, as well as at least two research assistants in the lab. Our philosophy is one of teamwork, a scholarly and critical attitude towards science and staying at the forefront of biomedical research approaches.